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Investigations into the immunological basis of eosinophilia induced by experimental schistosomiasis

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Antigen-specific granulomas about Schistosoma mansoni eggs, in normal or presensitized mouse lungs, contained more than 20% eosinophil leukocytes, in addition to macrophages, epithelioid cells, and lymphocytes. This was accompanied by blood eosinophilia. Delayed reactions to footpad tests with soluble egg antigen(s) showed histologic eosinophilia parallel with that in the pulmonary granulomas and blood. In sensitized guinea pigs, the inflammatory infiltration elicited by cercarial penetration of the skin, showed the characteristics of cutaneous basophil hypersensitivity while egg granulomas in the

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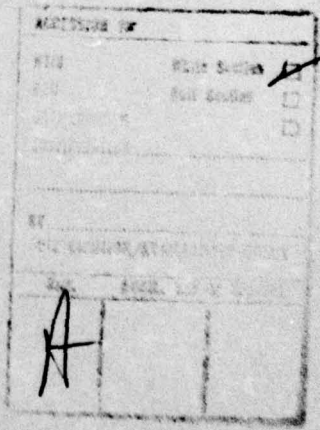
20. liver, like the murine egg granulomas, contained mononuclear cells and eosionphils.



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Contract termination report

Under this Army contract, the experiments conducted by the principal investigator have resulted in data necessitating a re-evaluation of the schistosome egg granuloma murine model first described by von Lichtenberg (1962) and defined by Warren and colleagues (see review of Warren, 1972). Although the granulomatous reaction about Schistosoma mansoni eggs in lungs appears to be a manifestation of cell mediated immunity or delayed hypersensitivity, significant differences do exist in comparison to other hypersensitivity granulomas, e.g. tubercle or fungal. My current studies demonstrate a significant proportion of the cells comprising the schistosome granuloma to be the eosinophilic polymorphonuclear leukocyte. Indeed, von Lichtenberg (1962) described eosinophils in such egg granulomata and in the arteritis that subsequently developed.

As described in the protocol of the original contract request (page 4), the time course of granuloma development and blood eosinophilia were determined in response to schistosome eggs. Control animals were injected initially with bentonite particles but subsequently a more suitable particle, Sepharose 4B beads (43 um to 71 um) were used. Minimal cellular reaction resulted around the latter particles in the lungs consisting of several macrophage-epithelioid type cells. However, when S. mansoni eggs were injected intravenously, granulomata formed about the eggs. Their size progressively increased to a maximum in approximately two weeks. Similar histopathologic findings were consistently found in four experiments with 40 B6D2 F1 mice.

In subsequent experiments, mice were sensitized by intraperitoneal injection of 2000 S. mansoni eggs or 2000 Sepharose 4B beads alone in phosphate-buffered saline. One week later, 10 mice in each group were injected intravenously with 2000 schistosome eggs and 10 mice in each group with 2000 Sepharose beads. At no time, covering a period from one day to 35 days after the intravenous injection of Sepharose beads, was there any cellular reaction about the beads. The injection of schistosome eggs in mice previously given Sepharose beads resulted in granulomata of typical size and composition as described above. But, in those mice given two injections of schistosome eggs, the granulomata sizes were larger and the maximum diameters occurred earlier, approximately one week after injection.

Ten experiments with a total of about 160 mice, control and experimental, resulted in consistent findings.

When these schistosome egg granulomata are analyzed as to the cell types involved, an interesting finding resulted. In confirmation of the detailed studies of Warren and colleagues (Warren, et al, 1967), mononuclear cells including lymphocytes, macrophages, and epithelioid cells are evident. But with the fixation employed in the present studies, Helly's fixative, and subsequent Giemsa staining, eosinophils are found to be as prominent in number as the aforementioned cell types. The presence of eosinophils were evident at all stages of the granulomatous inflammation after primary or secondary injections and usually numbered no fewer than 20 per cent of the cells. This observation of eosinophils in schistosome egg granulomata is in agreement with von Lichtenberg (1962) and Warren, et al, (1967) as to their presence but extends the phenomenon to suggest a more prominent role of the eosinophil in the granuloma's composition.

The histopathologic observations just described is supported by the presence of a progressive peripheral blood eosinophilia in mice intravenously injected with schistosome eggs. A representative experiment in table 1 shows that the mean number of eosinophils per c.mm. increased to a maximum by two to three weeks and slowly declined thereafter. No such rise was seen in mice exposed only to Sepharose 4B beads. Furthermore, blood eosinophilia was more marked when mice were presensitized with eggs and given a homologous challenge intravenously (table 2). The height of the eosinophilia occurred approximately one week earlier than that in mice given only one injection of schistosome eggs.

Efforts to induce blood eosinophilia by: 1) subcutaneous injection of up to 10,000 schistosome eggs; 2) intravenous injection of soluble egg antigens; and 3) intraperitoneal injection of eggs have, in general, been unsuccessful. However, the last named route did provide slight eosinophilia reaching a maximum in approximately three weeks (table 2) but did not appear to be statistically different from control groups of mice.

The observed eosinophilia and granulomata in response to schistosome eggs appeared to be antigenically specific in that presensitization of mice with 100 ug. bovine serum albumin or 20 ug.

Toxascaris leonina extract had no enhancing effect on the subsequent eosinophil levels or granuloma size.

Initial studies of serum taken from these various groups of mice have shown no anti-egg antibodies by immunodiffusion. However, further tests are in progress using the circumoval precipitin test, indirect hemagglutination test and passive cutaneous anaphylaxis test; this last mode to look for 7S gamma-1 and reaginic type antibodies.

As a presumptive test of delayed hypersensitivity, the footpad swelling test was employed. Groups of B6D2 F1 mice given schistosome eggs were footpad tested with 10 ug. soluble egg antigen at varying intervals from four days to 28 days. The opposite hind footpad was injected with phosphate-buffered saline. The volume injected in each footpad was 0.025 ml. and the 10 ug. dose was determined by preliminary trials with various concentrations in normal and egg sensitized mice. At varying times after injection, the footpad thicknesses were measured with a micrometer. At both 24 and 48 hours after footpad testing, the schistosome egg antigen injected footpad showed a mean increase of 0.25 to 0.35 mm. over the opposite control footpad. Positive results were found as early as four days after egg injection and remained so until the termination of the experiments at four weeks.

It would appear that related to the apparent delayed hypersensitivity nature of the schistosome egg granuloma is the eosinophil involvement in these lesions and the simultaneous blood eosinophilia on the basis of their simultaneous maximal responses and absence (?) of serum antibodies. Further effort is required to be sure of the absence of serum anti-egg antibodies.

At this time in these studies, I would suggest that the S. mansoni egg granuloma in the lungs is more a model of the egg granulomata observed in patent infections in mice by direct morphologic comparison of the cells involved, especially the eosinophil. Thus, this egg lesion should perhaps be called an eosinophil granuloma, more typical of helminth-induced eosinophil granulomata than of those induced by other infectious agents, e.g. fungi and tubercle bacilli.

Preliminary studies of the histopathology of the schistosome soluble egg antigen induced footpad swelling suggest further differences of this helminth model system compared to delayed hypersensitivity studies with other types of antigenic materials. While the positive 24-hour footpad test has been characterized as involving primarily

mononuclear cells, the present studies have found a large proportion of the infiltrating cells to be eosinophils. The appearance of eosinophils was not evident at 4 hours but consistently observed at 24 and 48 hours after testing. Recently, Dunsford, et al., (1974) described a predominantly mononuclear cell infiltrate in mouse footpads injected with schistosome egg antigen with rare neutrophils. The explanation for these differences is not readily apparent as the mode of sensitization was apparently the same, but the different strains of mice used and the fixation and staining differences may have contributed. However, in support of the present findings of local eosinophilia after antigen challenge is the observation that the high peripheral blood eosinophilia declined rapidly after footpad testing. The eosinophil levels were no different from normal levels after 24 hours. This phenomenon has not yet been described in the literature in relation to blood eosinophilia, footpad testing, and footpad eosinophilia in mice. The early literature on eosinophils has referred to a temporary drop in blood eosinophils upon subcutaneous or intraperitoneal injection of large doses of hydatid antigen in guinea pigs (Weinberg & Seguin, 1914). Studies are in progress to further delineate this phenomenon immunologically.

A companion study of schistosome infections in Hartley strain guinea pigs was conducted in collaboration with Dr. Philip W. Askenase, Department of Medicine, Yale University School of Medicine. We were able to demonstrate that the immune cellular response to cercarial penetration was primarily a form of cutaneous basophil hypersensitivity. It was further observed that the egg granulomata in the livers were composed of large numbers of eosinophils with mononuclear leukocytes. No basophils were observed. This pointed to a distinct difference in the leucocyte response to schistosomes depending on whether the skin was the site of reaction (basophils) or the viscera were the reacting tissues (eosinophils). This work has been recently published and the appropriate reprints are enclosed.

TABLE I

Blood eosinophil response to intravenous Schistosoma mansoni eggs in B6D2 F1 mice.

Day	Mean No. of Eosinophils per c.mm.	
	2000 Sepharose 4B beads (15 mice)	2000 S. mansoni eggs (15 mice)
0	156.8	147.2
4	195.2	258.7
7	133.3	284.8
14	277.3	545.1
21	229.3	497.1
28	246.4	462.9
35	256.0	465.1
42	240.0	367.7

TABLE 2

Comparison of blood eosinophil responses to intravenous Schistosoma mansoni eggs in B6D2 F1 mice previously sensitized with homologous eggs or Sepharose 4B beads, intraperitoneally.

		Mean Number of Eosinophils per c.mm.			
Day	Injection	Group 1	Group 2	Group 3	Group 4
	I.P.	2000 Sepharose 4B	2000 Sepharose 4B	2000 S. mansoni eggs	2000 S. mansoni eggs
0		134.4	121.6	102.4	171.2
4		156.8	136.0	112.0	208.0
7		134.4	124.8	249.6	321.6
	I.V.	Sepharose 4B	S. mansoni eggs	Sepharose 4B	S. mansoni eggs
11		206.8	211.2	382.0	363.2
14		144.0	360.0	435.2	1005.2
21		156.8	478.4	297.6	868.8
28		188.8	692.8	339.2	587.2
35		185.6	515.2	278.4	401.1
42		220.0	305.6	265.6	368.0
49		147.2	318.4	252.8	352.0
56		288.0	302.4	230.4	272.0

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